

# Wild Fisheries

## Spawning biomass of sardine, *Sardinops sagax*, in waters off South Australia in 2011



Ward, T.M., Ivey, A.R. and Burch, P.

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Report to PIRSA Fisheries and Aquaculture



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## **PREFACE**

The Daily Egg Production Method (DEPM) has been used to assess the stock status of sardine, *Sardinops sagax*, in South Australian waters since 1995. The estimate of spawning biomass obtained using this method the key biological performance indicator for the South Australian Sardine Fishery (SASF). Up until 2007, spawning biomass was estimated annually; since then the DEPM has been applied biennially. Since 2008, the Fishery Assessment Report, which integrates all information available for the South Australian Sardine Fishery, has been produced in the alternate year to the Spawning Biomass Report. The present report provides an estimate of the spawning biomass of sardine in waters off South Australia in February-March 2011.

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## EXECUTIVE SUMMARY

1. This report provides an estimate of the spawning biomass of sardine, *Sardinops sagax* in South Australian waters in 2011.
2. Data were obtained from research surveys conducted from the *RV Ngerin* during February and March 2011. The total survey area covered was 114,746 km<sup>2</sup>.
3. Sea surface temperatures (SSTs) during the surveys ranged from 15.6 to 25.8°C and were lowest in inshore waters off western Eyre Peninsula. SSTs in Gulf St Vincent, Spencer Gulf and Investigator Strait in 2011 were generally higher than in previous years, whereas chlorophyll-a concentrations and zooplankton densities were relatively low across the entire survey.
4. A total of 2,486 live sardine eggs were collected from 339 stations. High densities of eggs were recorded south of Spencer Gulf, across the mid shelf and inshore regions west of Eyre Peninsula and south of Kangaroo Island.
5. A total of 14 samples comprising 1,917 mature fish was collected at sampling locations in Investigator Strait, southern Spencer Gulf and the eastern Great Australian Bight.
6. The estimated total spawning area (*A*) in 2011 was 42,075 km<sup>2</sup>.
7. Mean daily egg production (*P<sub>0</sub>*), calculated using the log-linear version of the egg mortality model, was 44.0 eggs.day<sup>-1</sup>.m<sup>-2</sup> (95% CI = 27.8 – 71.1 eggs.day<sup>-1</sup>.m<sup>-2</sup>).
8. Estimates of mean adult reproductive parameters in 2011 were: female weight, *W* = 46.6 g (95% CI = 42.3 – 51.2); batch fecundity, *F* = 16,049 (95% CI = 13,649 – 18,806) hydrated oocytes, sex ratio, *R* = 0.63 (95% CI = 0.55 – 0.73) and spawning fraction, *S* = 0.044 (95% CI = 0.031 – 0.058).
9. The best estimate of spawning biomass for 2011 was 193,201 t (95% CI = 104,151–381,961), which lies within the target range of spawning biomass of 150,000-300,000 t.

## 1. INTRODUCTION

### 1.1 Daily Egg Production Method

The Daily Egg Production Method (DEPM) was developed for stock assessment of the northern anchovy, *Engraulis mordax* (Parker 1980; Lasker 1985) and has been applied to at least 18 species of small pelagic fishes worldwide (Stratoudakis et al. 2006; Neira et al. 2008; Dimmlich et al. 2009; Ward et al. 2009a). The method is widely used because it is often the most practical option available for stock assessment of small pelagic species. In many circumstances the only real alternative to the DEPM is acoustic surveys, which can produce biased estimates of biomass and require more sophisticated and expensive infrastructure, higher levels of technical support and expertise and have a longer developmental phase than the DEPM. A good example of these constraints comes from South Africa, where estimates of anchovy spawner biomass obtained using the DEPM over a decade were used to scale-up negatively-biased acoustic estimates while methods were being developed for *in situ* estimation of acoustic target strength (Hampton 1996).

The DEPM relies on the premise that the biomass of spawning adults can be calculated by dividing the mean number of pelagic eggs produced per day throughout the spawning area, i.e. total daily egg production, by the mean number of eggs produced per unit mass of adult fish, i.e. mean daily fecundity (Lasker 1985). Total daily egg production is the product of mean daily egg production ( $P_0$ ) and total spawning area ( $A$ ). Mean daily fecundity is calculated by dividing the product of mean sex ratio (by weight,  $R$ ), mean batch fecundity (number of oocytes in a batch,  $F$ ) and mean spawning fraction (proportion of mature females spawning each day/night,  $S$ ) by mean female weight ( $W$ ). Spawning biomass ( $SB$ ) is calculated according to the equation:

$$SB = P_0 \cdot A / (R \cdot F \cdot S / W). \quad \text{Equation 1}$$

The DEPM can be applied to fishes that spawn multiple batches of pelagic eggs over an extended spawning season (e.g. Parker 1980). Data used to estimate DEPM parameters are typically obtained during fishery-independent surveys involving vertical plankton tows at sites located at regular intervals along parallel cross-shelf transects. Adult samples are often taken opportunistically during the survey and may be complemented by samples collected concurrently from commercial vessels (Stratoudakis et al. 2006). The key assumptions of the DEPM are that: 1) surveys are conducted during the main (preferably peak) spawning season; 2) the entire spawning area is sampled; 3) eggs are sampled without loss and identified without error; 4) levels of egg production and mortality are consistent across the spawning area; and 5) representative samples of spawning adults are collected during the survey period (Parker 1980; Alheit 1993; Hunter and Lo 1997; Stratoudakis et al. 2006).

Although the DEPM is used widely, a range of problems have been encountered, and estimates of spawning biomass are generally considered to be accurate (unbiased) but relatively imprecise (e.g. Alheit 1993; Hunter and Lo 1997; Stratoudakis et al. 2006). This imprecision is mainly due to uncertainties associated with the estimation of total daily egg production, i.e.  $P_0$  and  $A$ . (Fletcher et al. 1996; McGarvey and Kinloch 2001; Ward et al. 2001a; Gaughan et al. 2004). A range of analytical methods have been used to calculate these parameters and these have the potential to significantly affect estimates of SB. For example, egg age has been estimated using a range of models that combine information on daily spawning synchronicity and mean egg developmental rates in relevant temperature ranges (e.g. Lo 1985; Piquelle and Stauffer 1985; Ibaibarriaga 2007). Perhaps most importantly,  $P_0$  has been determined by fitting the exponential decay model to estimates of the mean age of daily cohorts and their density in each sample using non-linear regression, a log-linear model of ln-transformed data (e.g. Piquelle and Stauffer 1985), generalised linear models (GLM) with appropriate link functions and generalised additive models (GAM) (ICES 2004). The delta method (Seber 1982) and parametric (Borchers et al. 1997) and non-parametric bootstraps (Jackson and Chen 2001; ICES 2004) have been used to estimate confidence intervals of  $P_0$ .  $A$  has been estimated by dividing the survey into stratified grids using a subjective 'manual' method (Lasker 1985) and objectively using nearest neighbour methods (Watson 1981; Ward et al. 2009a). Confidence intervals for  $A$  have been estimated using GAMs (Stratoudakis et al. 2003).

Many DEPM studies have been impeded by difficulties associated with obtaining representative samples of adults to estimate reproductive parameters (see Stratoudakis et al. 2006). Estimating  $R$  and  $W$  is relatively straightforward if representative samples can be collected.  $F$  has been estimated by calculating the relationship between fish weight (ovary-free) and batch fecundity for females with gonads containing hydrated oocytes using either linear regression (Piquelle and Stauffer 1985) or a gamma or negative binomial GLM with an identity link function (ICES 2004); this relationship has then been applied to the mean gonad free weight of all mature female fish.  $S$  is often the most difficult DEPM parameter to estimate for clupeoids. Obtaining representative samples of adults is difficult because during the spawning period spawning females are over-represented in ephemeral spawning aggregations and under-represented in the remainder of the population (Stratoudakis et al. 2006). Much of the uncertainty surrounding estimates of  $S$  is associated with determining whether imminent or recent spawners or both should be used in calculations.

At least two reviews have concluded that the DEPM may be better tailored to anchovies (*Engraulis* spp.) than to sardine, *Sardinops sagax* (Alheit 1993; Stratoudakis et al. 2006). The main argument used to support this assertion is that because a higher proportion of anchovies are actively

spawning during the peak spawning season, daily fecundity can be estimated more precisely for anchovy than sardine (e.g. Alheit 1993; Stratoudakis et al. 2006). Despite these apparent limitations of the DEPM for stock assessment of sardine, the method is a critical component of the assessment of this species in several locations. For example, the DEPM has been used for stock assessment of *S. sagax* off the west coast of North America (e.g. Lo et al. 2005) and the western and southern coasts of Australia (Fletcher et al. 1996; Gaughan et al. 2004; Ward et al. 2009b). However, there are important differences between locations in the manner in which egg and adult samples are collected, data are analysed and in how estimates of spawning biomass are used to support fisheries management. A Continuous Underway Fish Egg Sampler has been used routinely in surveys off California but not Australia (e.g. Lo et al. 2001). In addition, adult samples have usually been collected by mid-water trawling off California, purse-seining off Western Australia (Gaughan et al. 2004) and gill-netting off South Australia (Ward et al. 2009b). Furthermore, estimates of *SB* obtained from DEPM surveys are used directly for fisheries management in South Australia, but are incorporated into age-structured stock assessment models in California and Western Australia.

## **1.2 Application of the DEPM off South Australia**

The DEPM has been used to estimate the spawning biomass of sardine, *Sardinops sagax*, in South Australian (SA) waters since 1995 (Ward et al. 1998, 2009b). Application of this method has facilitated the rapid and sustainable development of the South Australian Sardine Fishery (SASF), despite the effects of two mass mortality events that both killed over 70% of the adult population of sardine in SA waters (e.g. Ward et al. 2001a, 2008). The current harvest strategy indicates that a baseline TACC of 30,000 t will be maintained while the latest estimate of spawning biomass remains between 150,000 and 300,000, which corresponds to exploitation rates of 20% and 10%, respectively. Since 2010 an additional 4,000 t has been allocated to be caught outside traditional fishing areas. A recent review that reanalysed data collected since 1998 using the various statistical methods identified the optimal approach for applying the DEPM in SA waters (Ward et al. 2011). That review, and a study that investigated the potential for utilising a CUFES in future studies (Ward and Ivey 2009), also identified options for improving the methods currently used to estimate key DEPM parameters.

## **1.3 Aim and Objectives**

This report provides an estimate of the spawning biomass of sardine in gulf and shelf waters of SA during February-March 2011. The objectives of the report are:

1. To describe the distribution and abundance of sardine eggs in relation to environmental factors;
2. To estimate DEPM parameters (*A*, *P*, *W*, *R*, *F*, *S*) and
3. To use the DEPM to estimate the spawning biomass in 2011

## 2. METHODS

### 2.1 Study Area and Biophysical Variables

#### 2.1.1 Study area

Two surveys were conducted aboard the *RV Ngerin* in shelf and gulf waters of South Australia between February and March 2011. Plankton samples were collected at 340 stations on 34 transects between Victor Harbor and Head of Bight (Fig. 1).

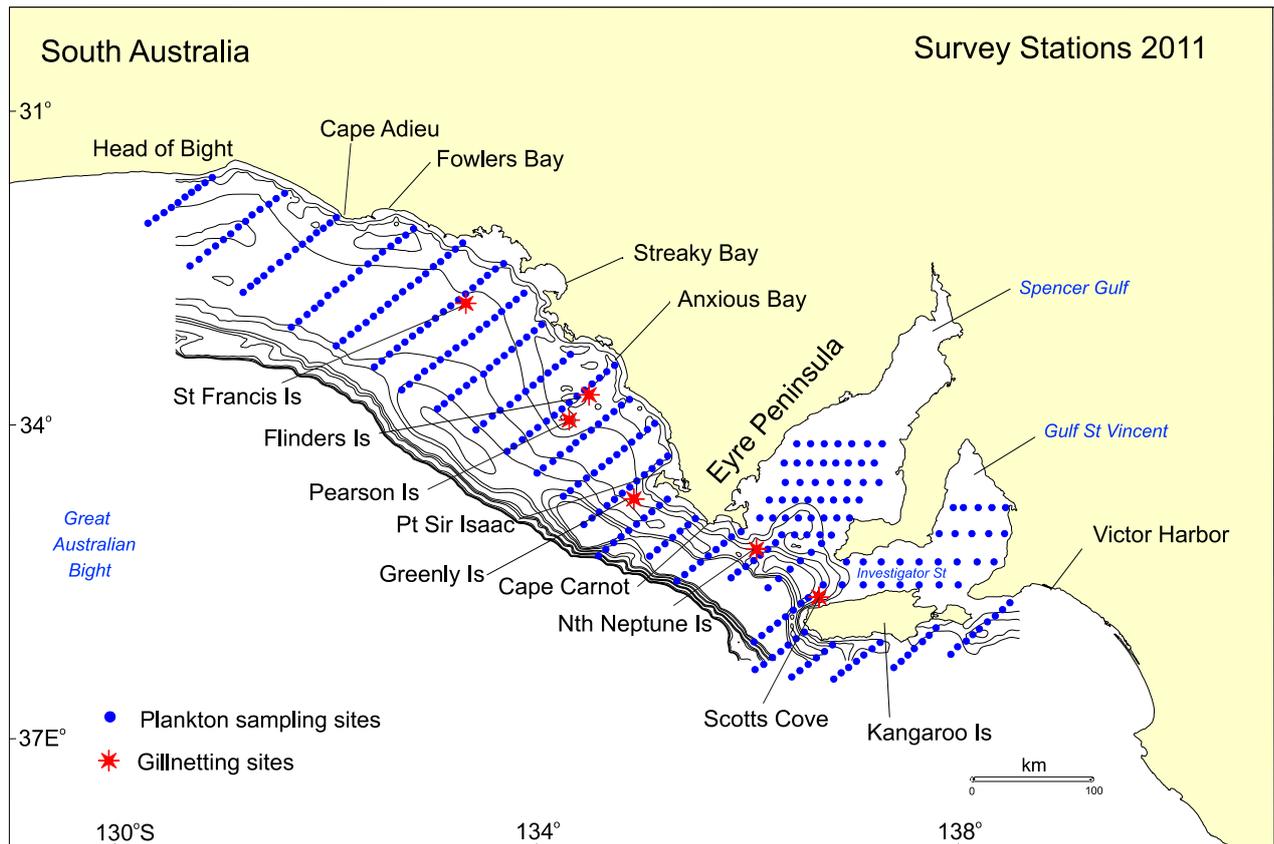


Figure 1. Map of South Australia showing stations where plankton and adult samples were collected during the 2011 DEPM surveys.

#### 2.1.2 Water temperature and primary production

At each station (Fig. 1), a *Sea-Bird* Conductivity-Temperature-Depth (CTD) recorder fitted with a fluorometer was lowered to a depth of 70 metres, or to 10 metres from the bottom in waters less than 80 m deep. Estimates of water temperature and fluorescence at a depth of 3 m were extracted from each profile. Where CTD temperature was absent a correction factor was applied to the on-board temperature measurement (average difference between good CTD and on-board temperature). Fluorescence is an indicator of primary production and gives an un-calibrated measure of chlorophyll-a concentration ( $\mu\text{g.L}^{-1}$ ). Spatial plots of SST and chlorophyll-a concentration were prepared using minimum curvature algorithms in Surfer<sup>®</sup> (Ver. 8).

### 2.1.3 Secondary production – zooplankton abundance

An index of zooplankton abundance at each station was estimated by dividing the displacement volume of zooplankton (ml) collected during plankton tows by the total volume of water filtered ( $m^3$ ). Spatial plots of zooplankton abundance were prepared using minimum curvature algorithms in Surfer® (Ver. 8).

## 2.2 Daily Egg Production and Spawning Area

### 2.2.1 Plankton sampling

Plankton samples were collected at each station using paired Californian Vertical Egg Tow (CalVET) plankton nets. Each CalVET net had an internal diameter of 0.3 m, 330  $\mu m$  mesh and plastic cod-ends. During each tow the CalVET nets were deployed to within 10 m of the seabed at depths <80 m or to a depth of 70 m at depths >80 m and retrieved vertically at a speed of  $\sim 1 m.s^{-1}$ . General Oceanics™ 2030 flow-meters and factory calibration coefficients were used to estimate the distance travelled by the net during each tow. Where there was a discrepancy of more than 500 units between flowmeters, the relationship between wire length released and flow-meter units was used to determine which was correct and that value repeated. Upon retrieval of the nets the samples from each of the two cod-ends were washed into a sample container. Plankton samples were fixed using 5% buffered formaldehyde and seawater.

### 2.2.2 Laboratory analysis

Sardine eggs and larvae were identified in each sample using published descriptions (White and Fletcher 1996; Neira et al. 1998). Eggs in each sample were counted, staged and assigned approximate ages based on descriptions and temperature-development keys in White and Fletcher (1996).

### 2.2.3 Egg density

The number of eggs of each stage under one square metre of water ( $P_t$ ) was estimated at each site according to equation 2:

$$P_t = \frac{C \cdot D}{V} \qquad \text{Equation 2}$$

where  $C$  is the number of eggs of each age in each sample,  $V$  is the volume filtered ( $m^3$ ), and  $D$  is the depth (m) to which the net was deployed (Smith and Richardson 1977). Plots of egg distribution and abundance were prepared using Surfer® (Ver. 8).

#### *2.2.4 Spawning time and density weightings*

The development time of sardine eggs is known to be dependent on water temperature (Picquelle and Stauffer 1985). Kernel density methods were used to estimate the modal time of egg abundance for three categories of SST recorded in South Australian waters (Ward et al. 2011). A peak spawning time of 2:00 am was assumed based on the assumption that Stage 2 eggs are approximately 3-4 hours old. In waters <19.0°C, 19.0-20.0°C and >20.0°C, Stages 1-6, 1-7 and 1-8 were less than 24 hours old and Stage 7-12, 8-12 and 9-12 eggs were 24-48 hours old. Ages were assigned to day-1 eggs (i.e. 0 – 24 hours old) by subtracting the estimated spawning time from the sampling time. Ages of day-2 eggs were assigned similarly, but an additional 24 hours were added to their ages. Densities of day-1 and day-2 eggs were weighted according to the relative size of the area from which they were taken.

#### *2.2.5 Spawning area*

The Voronoi natural neighbour (VNN) method (Watson 1981) in Mapinfo® (Vers. 8) was used to generate a polygon around each sampling site with the boundary as the midpoint equidistant between each sampling site (Fig. 2). The area represented by each station (km<sup>2</sup>) was then determined. The spawning area (*A*) was defined as the total area of grids where live sardine eggs were found.

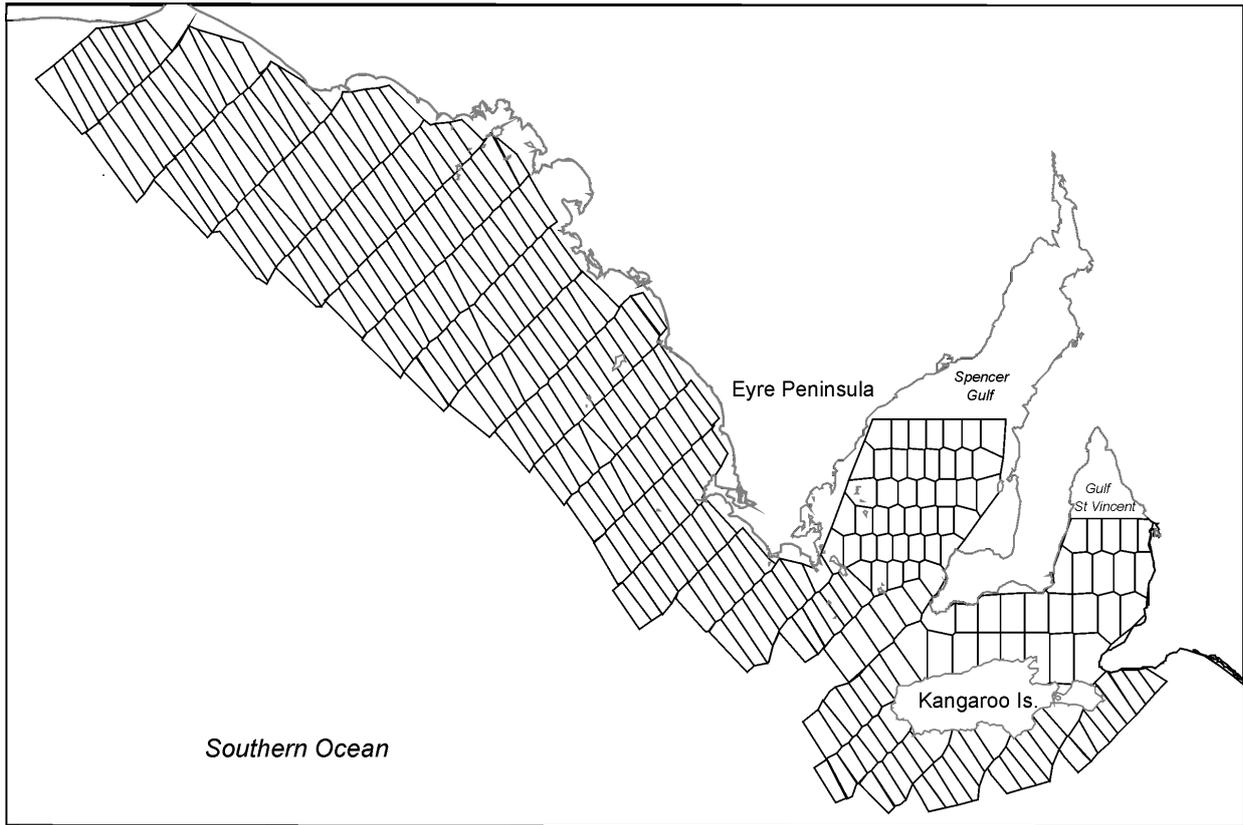


Figure 2. Voronoi nearest neighbour polygons generated in Mapinfo® (vers. 8) used to estimate the total spawning area in 2011.

### 2.2.6 Daily egg production ( $P_0$ ) and egg mortality

Biased mean daily egg production ( $P_b$ ) was calculated by fitting the linear version of the exponential egg mortality model to estimates of egg age and density at each station (Picquelle and Stauffer 1985). To allow the inclusion of data from stations where either day 1 or day 2 eggs were absent, one egg was added to the counts of both day 1 and day 2 eggs at every positive station. The linear version of the exponential egg mortality model is:

$$\ln P_b = \ln(P_i) - Zt \quad \text{Equation 3}$$

where  $P_i$  is the density of eggs of age  $t$  at site  $i$  and  $Z$  is the instantaneous rate of egg mortality.

Estimates of  $P_b$  obtained using the linear version of the exponential mortality model have a strong negative bias, therefore a bias correction factor was applied following the equation of Picquelle and Stauffer (1985):

$$P_0 = e^{(\ln P_b + \sigma^2/2)} \quad \text{Equation 4}$$

where  $\sigma^2$  is the variance of the estimate of biased mean daily egg production ( $P_b$ ).

## 2.3 Adult Reproductive Parameters

### 2.3.1 Sampling methods

Each afternoon areas where sardine schools were known to aggregate and were suitable for gillnetting (i.e. adequately protected from the swell) were searched using a dual frequency echo sounder (*Furuno* - 60 and 180 KHz) (Fig. 1). The *RV Ngerin* was then anchored where several schools were observed. Samples of adults were collected using a gillnet comprising three panels, each with a different multi-filament nylon mesh size (*double diamond*: 210/4 ply meshes – 25, 28 and 32 mm). Surface and sub-surface lights (500 W) were illuminated near the net after it was set. Net soak times varied from 15 minutes to 3 hours depending on the number of fish caught. After the net was retrieved, fish were removed and dissected immediately. Mature and immature males and females were counted. Mature females were fixed in 5% buffered formaldehyde solution. Immature females and males were frozen. Calculations of female weight, sex ratio, batch fecundity and spawning fraction were based on samples collected from Scotts Cove in Investigator Strait, North Neptune Island in southern Spencer Gulf and Greenly, Pearson and St Francis Island in the eastern Great Australian Bight.

### 2.3.2 Female weight ( $W$ )

Mature females from each sample were removed from formalin and weighed ( $\pm 0.01$  g). Fixation in formalin has a negligible effect on fish weight (Lasker 1985). The mean weight of mature females in the population was calculated from the average of sample means weighted by proportional sample size:

$$W = \left[ \overline{W}_i * \frac{n_i}{N} \right] \quad \text{Equation 5}$$

where  $\overline{W}_i$  is the mean female weight of each sample  $i$ ;  $n$  is the number of fish in each sample and  $N$  is the total number of fish collected in all samples.

### 2.3.3 Male weight

Mature males in each sample were thawed and weighed ( $\pm 0.01$  g).

#### 2.3.4 Sex ratio ( $R$ )

The mean sex ratio of mature individuals in the population was calculated from the average of sample means weighted by sample size:

$$R = \left[ \overline{R}_i * \frac{n_i}{N} \right] \quad \text{Equation 6}$$

where  $n$  is the number of fish in each sample,  $N$  is the total number of fish collected in all samples and  $\overline{R}_i$  is the mean sex ratio of each sample calculated from the equation:

$$\overline{R}_i = \frac{F}{(F + M)} \quad \text{Equation 7}$$

where  $F$  and  $M$  are the respective total weights of mature females and males in each sample  $i$ . The mean sex ratio was also calculated for all years data from 1998 – 2011.

#### 2.3.5 Batch fecundity ( $F$ )

Batch fecundity was estimated from ovaries containing hydrated oocytes using the methods of Hunter *et al.* (1985). Both ovaries were weighed and the number of hydrated oocytes in three ovarian sub-sections were counted and weighed. The total batch fecundity for each female was calculated by multiplying the mean number of oocytes per gram of ovary segment by the total weight of the ovaries. The relationship between female weight (ovaries removed) and batch fecundity was determined by linear regression analysis and used to estimate the batch fecundities of mature females in all samples.

#### 2.3.6 Spawning fraction ( $S$ )

Ovaries of mature females were sectioned and stained with haematoxylin and eosin. Several sections from each ovary were examined to determine the presence/absence of post-ovulatory follicles (POFs). POFs were aged according to the criteria developed by Hunter and Goldberg (1980) and Hunter and Macewicz (1985). The spawning fraction of each sample was estimated as the mean proportion of females with hydrated oocytes plus day-0 POFs ( $d0$ ) (assumed to be spawning or have spawned on the night of capture), day-1 POFs ( $d1$ ) (assumed to have spawned

the previous night) and day-2 POFs ( $d2$ ) (assumed to have spawned two nights prior). The mean spawning fraction of the population was then calculated from the average of sample means weighted by proportional sample size.

$$S = \left[ \overline{S_i} * \frac{n_i}{N} \right] \quad \text{Equation 8}$$

where  $n$  is the number of fish in each sample,  $N$  is the total number of fish collected in all samples and  $\overline{S_i}$  is the mean spawning fraction of each sample calculated from the equation:

$$\overline{S_i} = \frac{[(d0 + d1 + d2POFs) / 3]}{n_i} \quad \text{Equation 9}$$

where  $d0$ ,  $d1$  and  $d2$  POFs are the number of mature females with POFs in each sample and  $n_i$  is the total number of females within a sample.

## 2.4 Spawning Biomass and bootstrapping procedures

### 2.4.1 Spawning biomass estimates

Estimates of spawning biomass were calculated according to equation 1 using the estimate of  $P_0$  obtained via the log-linear model and from adult reproductive parameters collected during:

- the 2011 survey;
- all data since 1998 to estimate sex ratio ( $R$ );
- spawning fraction ( $S$ ) 1.5 times the value determined in 2011;

### 2.4.2 Bootstrapping procedures and confidence intervals

To account for the covariance of adult parameters within individual samples, confidence intervals for all four adult parameters were calculated using a two stage bootstrap with 100,000 bootstrap iterations (Efron and Tibshirani 1993). For each iteration, the individual samples were resampled with replacement to obtain the bootstrapped samples. For each of the bootstrapped samples, the fish were resampled with replacement to generate a complete survey. The adult parameters  $W$ ,  $S$  and  $R$  were calculated from the bootstrapped survey using the method described above. Batch fecundity ( $F$ ) was calculated from the mean gonad-free weight using the batch relationship obtained by bootstrapping with replacement from females with hydrated oocytes. For each bootstrap iteration the value  $W / R.F.S$  was used in the calculation of bootstrapped confidence

intervals for spawning biomass. The 95% confidence intervals of spawning biomass were estimated by calculating the spawning biomass 100,000 times from  $A$  and the 100,000 bootstrapped estimates of  $P_0$  and  $W/R.F.S$  using the percentile method. Parameter estimates were calculated independently in Excel 2003 and R 2.9.2 with confidence intervals estimated with R 2.9.2.

### 3. RESULTS

#### 3.1. Biophysical variables

##### 3.1.1 Sea surface temperature

Sea surface temperatures (SSTs) ranged from 15.6 to 25.8°C (Fig. 3) during February and March 2011. Low SSTs (< 18°C) were recorded in inshore water along the western coast of Eyre Peninsula. High SSTs (> 19°C) were recorded in Spencer Gulf, Gulf St Vincent, south of Kangaroo Island and throughout the central Great Australian Bight.

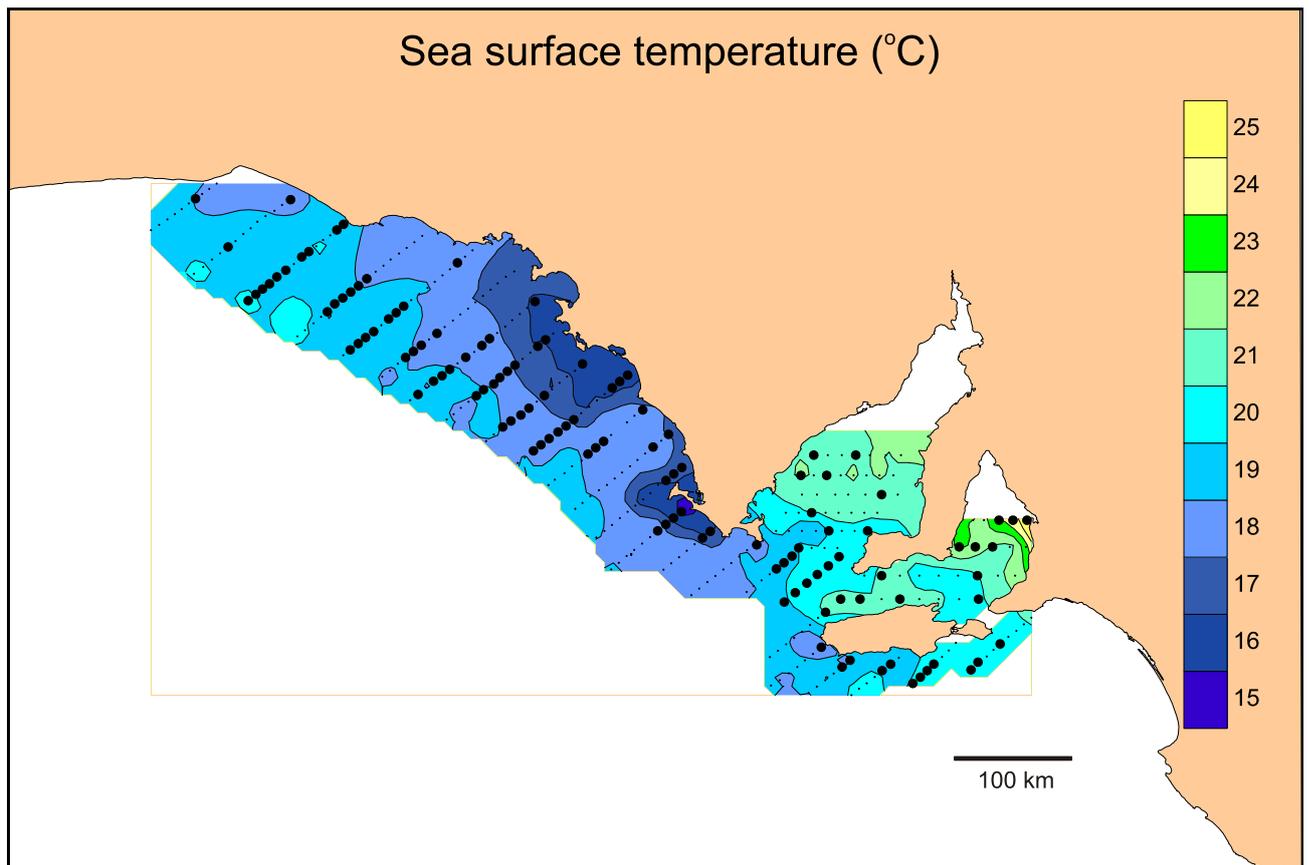


Figure 3. Sea-surface temperature profile across the 2011 February – March survey, showing stations where sardine eggs were collected (●).

### 3.1.2 Fluorescence (chlorophyll-a)

Chlorophyll-a concentration at each station ranged between 0.003 and 0.72  $\mu\text{g.L}^{-1}$  (Fig. 4) during February and March 2011. The highest values were recorded off Coffin Bay Peninsula, Anxious Bay and south east of Kangaroo Island. The remainder of coastal and shelf waters mainly had chlorophyll-a concentrations ranging between 0.01 and 0.2  $\mu\text{g.L}^{-1}$ .

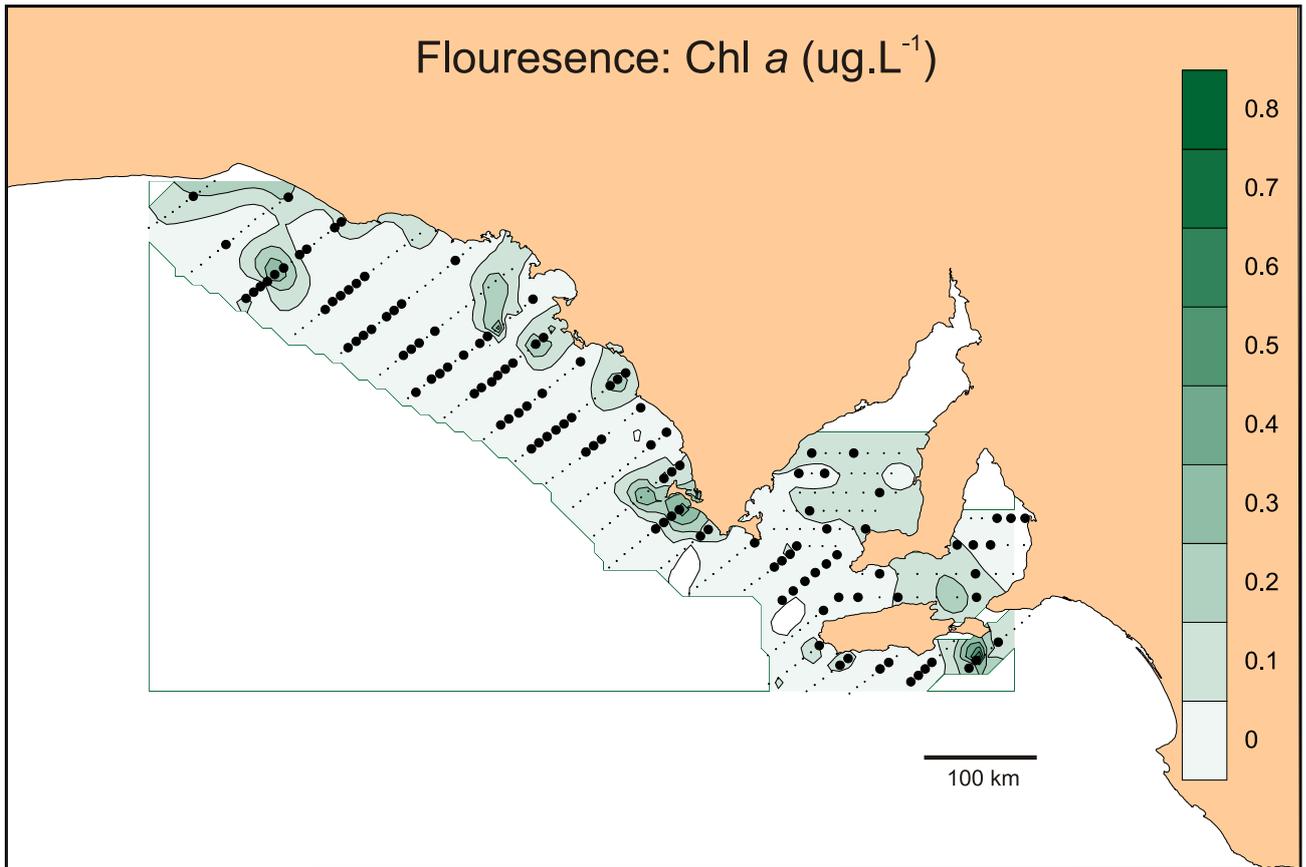


Figure 4. Surface concentration of chlorophyll-a inferred from fluorecence readings at 3 m across the 2011, February - March survey area, showing stations where sardine eggs were collected (●).

### 3.1.3 Zooplankton abundance

Total densities of zooplankton ranged between 0.08 and 12.8 ml.m<sup>-3</sup> (Fig. 5) during February and March 2011. The patches of zooplankton taxa observed on the outer shelf off western Eyre Peninsula were comprised mostly of salps. The highest densities of small zooplankton taxa were found in the mouth of Spencer Gulf, Investigator Strait, inshore west of Eyre Peninsula, south of Kangaroo Island and near Cape Adieu.

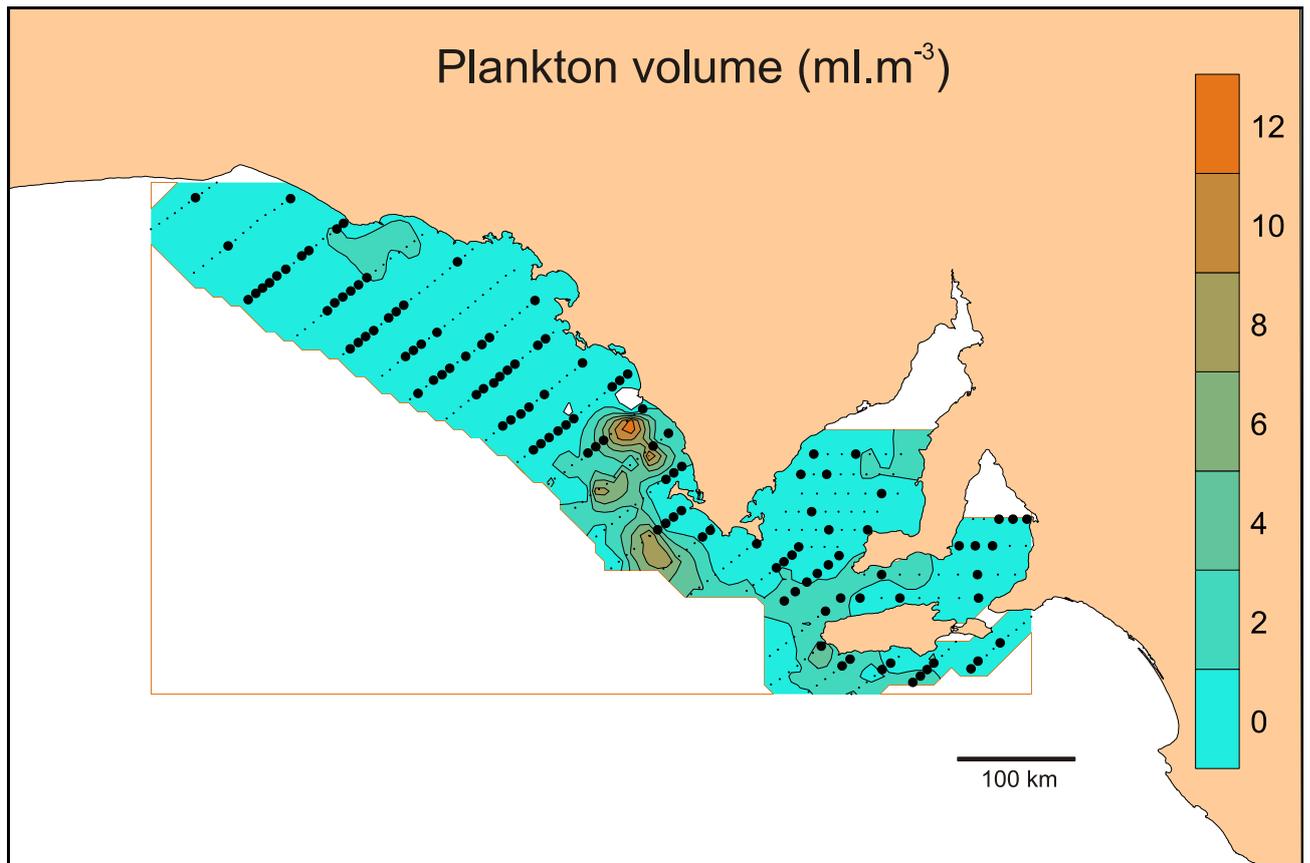


Figure 5. Distribution and abundance (ml.m<sup>-3</sup>) of zooplankton across the 2011, February - March survey area, showing stations where sardine eggs were collected (●).

### 3.2 Distribution and Abundance of Eggs and Larvae

#### 3.2.1 Distribution and abundance of eggs

A total of 2,486 live sardine eggs were collected at 121 of 340 (35.6%) stations on 34 transects between the Head of Bight and Victor Harbor (Fig. 6) during February and March 2011. The stations with the highest egg densities were located in the mouth of Spencer Gulf, north of Point Sir Isaac, south of Kangaroo Is. and on the mid shelf region of the eastern part of the Great Australian Bight. Egg densities up to 6,616 eggs.m<sup>-2</sup> were recorded in these regions.

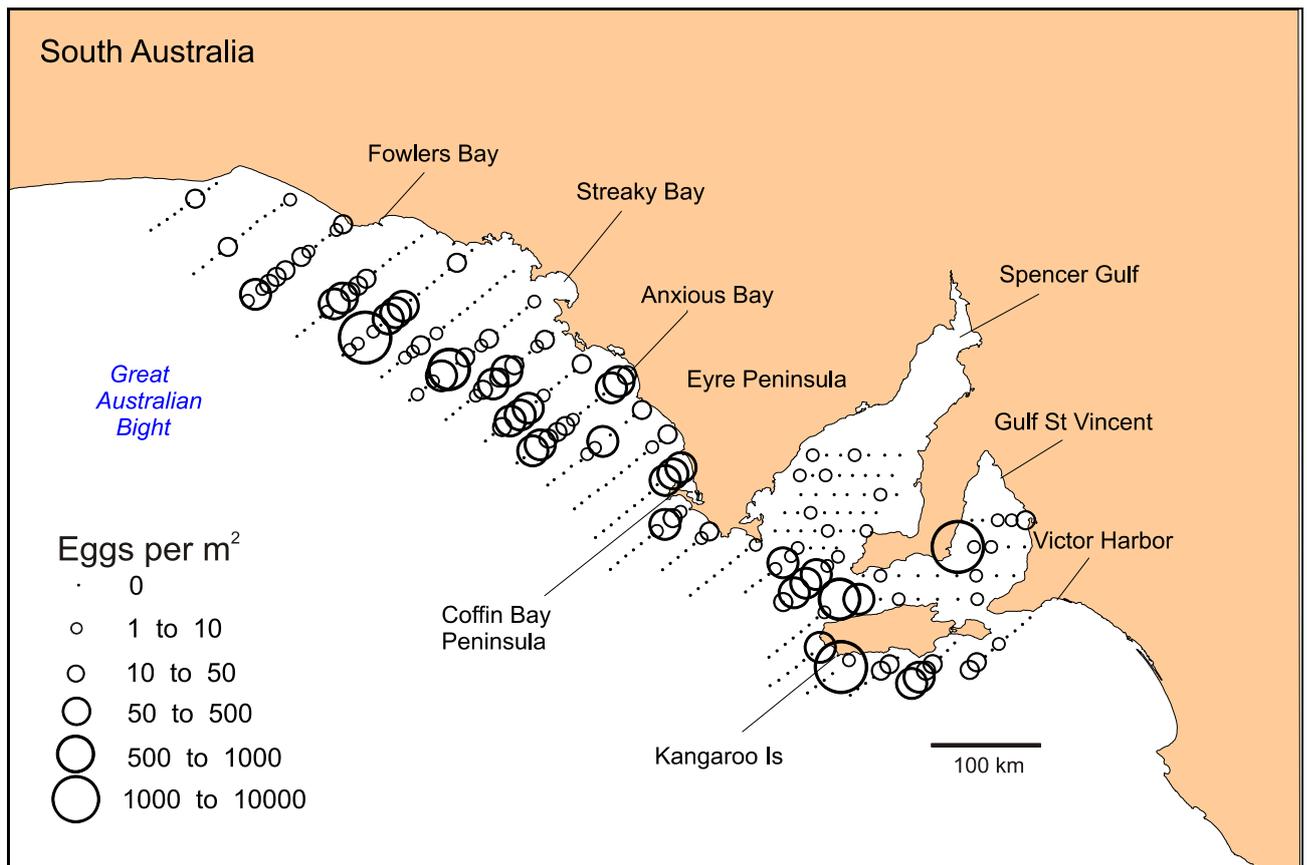


Figure 6. Spatial patterns of live sardine egg distribution and abundance during February and March 2011.

### 3.2.2 Larval abundance and distribution

A total of 2,119 sardine larvae were collected at 181 of 339 stations (53.4%) between the Head of Bight and Victor Harbor (Fig. 7) during February and March 2011. The spatial distribution of larvae was similar to that of sardine eggs, although more widespread. Densities were highest west of Venus Bay, adjacent to Coffin Bay Peninsula, in southern Spencer Gulf, and south of Kangaroo Island, and ranged between 5 and 534 larvae.m<sup>-2</sup>.

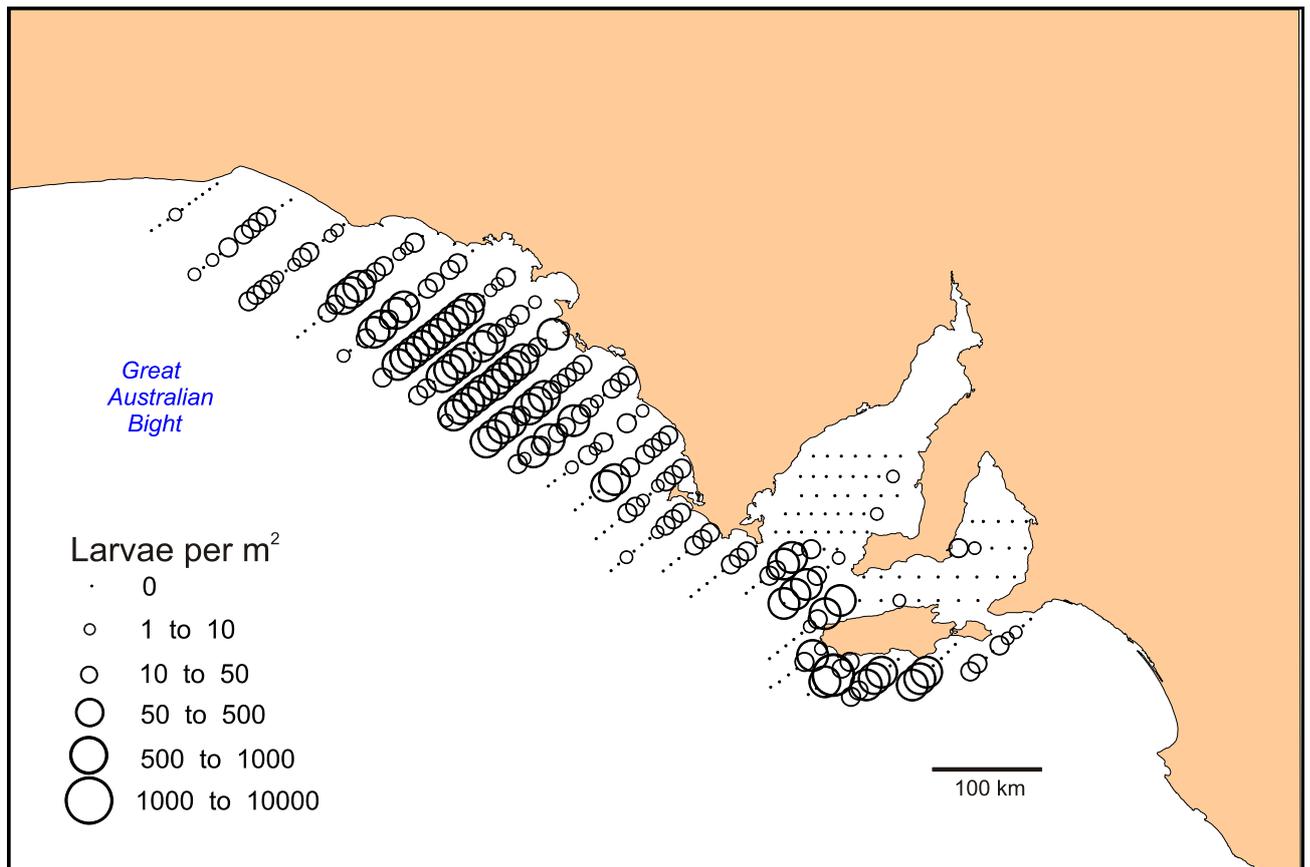


Figure 7. Spatial patterns of sardine larval distribution and abundance during February and March 2011.

### 3.3 Spawning Area

The estimated spawning area for the entire survey area was 42,075 km<sup>2</sup>, comprising 36.7% of the total area sampled (114,746 km<sup>2</sup>) (Table 1).

**Table 1.** Mean daily egg production ( $P_0$ , log-linear model), spawning area ( $A$ ) and spawning biomass. Table shows the variance ( $\sigma^2$ ) term used in estimate.

	Area sampled (km <sup>2</sup> )	Spawning area $A$ (km <sup>2</sup> )	Percentage of area sampled	$\sigma^2 P_b$	$P_0$ (eggs.d <sup>-1</sup> .m <sup>-2</sup> )	Spawning biomass (t)
Total survey	114,745	42,075	36.7	1.47	43.95	193,210

### 3.4 Daily Egg Production ( $P_0$ )

The estimate of mean daily egg production,  $P_0$  obtained using the linear version (Eq. 3) of the exponential egg mortality (recommended by Ward et al. 2011) was 44.0 eggs.day<sup>-1</sup>.m<sup>-2</sup> (95% CI = 27.8 – 71.1, Fig. 8, Table 1,5).

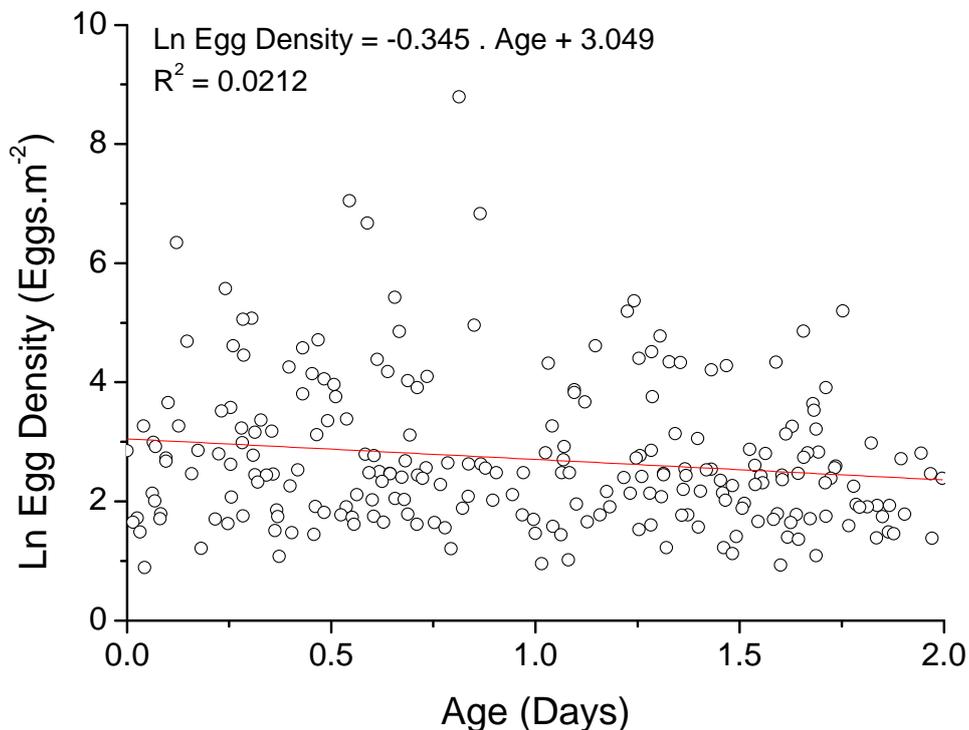


Figure 8. Linear regressions between ln-transformed sardine egg density (eggs.m<sup>-2</sup>) and age (days) data in 2011.

### 3.5 Adult Reproductive Parameters

A total of 14 samples comprising 1870 mature sardines were collected at Scotts Cove, St Francis Is., Flinders Is., Pearson Is. and North Neptune Is. during the 2011 survey (Table 2). Estimates of the adult female reproductive parameters used in calculations of spawning biomass are provided in Tables 3, 4 and 5. The ranges of adult parameters calculated from samples collected between 1998 and 2011 are provided in Table 5. Bootstrapped parameter estimates that provided 95% confidence intervals are shown in Table 5.

**Table 2.** Sampling details for adult sardine collected in Investigator Strait and the eastern Great Australian Bight during the 2011 DEPM surveys.

Date	Location	Survey	N samples	<i>n</i> fish
04/02/2011	Scotts Cove	1	3	409
12/02/2011	Scotts Cove	1	3	472
09/03/2011	St Francis Is.	2	1	118
10/03/2011	Pearson Is.	2	3	589
11/03/2011	Flinders Is.	2	1	50
12/03/2011	N. Neptune Is.	2	1	85
13/03/2011	N. Neptune Is.	2	2	194
		Total	14	1917

#### 3.5.1 Mean female weight

The mean weight of mature females in samples ranged from 36.5 to 60.4 g (Table 3). The weighted mean weight of mature females in 2011 was 46.6 g (95% CI = 42.3 – 51.2, Table 3, 5).

#### 3.5.2 Sex ratio

The sex ratio calculated from the 2011 survey was the highest observed since 1998 (0.63, 95% CI = 0.55 – 0.73) (Table 5). The mean sex ratio from data collected between 1998 and 2011 was 0.52 and ranged between 0.36 and 0.63 (Table 5).

**Table 3.** Number of sardine in samples by sex and estimates of female weight,  $W$  and sex ratio,  $R$  (proportion of females by weight) for samples collected in 2011. Values in bottom row are sums (\*) and weighted means (#). Note males from sample 7 were lost and this sample was excluded from ratio calculation.

Sample	Location	Date	Male	Female	Mean Male Weight	Mean Female Weight ( $W$ )	Sex Ratio by weight ( $R$ )
1	Scotts Cove	04/02/2011	52	90	36.3	43.1	0.67
2	Scotts Cove	04/02/2011	43	86	35.7	38.9	0.68
3	Scotts Cove	04/02/2011	49	89	35.2	40.1	0.67
4	Scotts Cove	12/02/2011	88	89	39.2	45.3	0.54
5	Scotts Cove	12/02/2011	48	95	47.5	52.1	0.68
6	Scotts Cove	12/02/2011	64	88	46.6	50.0	0.60
7	St Francis Is.	09/03/2011	47	71	N/A	46.9	N/A
8	Pearson Is.	10/03/2011	93	96	36.0	37.7	0.52
9	Pearson Is.	10/03/2011	90	91	33.5	36.5	0.52
10	Pearson Is.	10/03/2011	127	92	38.9	39.3	0.42
11	Flinders Is.	11/03/2011	18	32	41.7	45.2	0.66
12	N. Neptune Is.	12/03/2011	11	74	51.4	58.0	0.88
13	N. Neptune Is.	13/03/2011	4	90	66.0	59.7	0.95
14	N. Neptune Is.	13/03/2011	8	92	58.1	60.4	0.92
			<b>695*</b>	<b>1175*</b>	<b>39.1#</b>	<b>46.6#</b>	<b>0.63#</b>

**Table 4.** Number of female sardine in samples and estimates of spawning fraction ( $S$ ) for samples collected in 2011. Values in bottom row are sums\* and weighted means#.

Sample	Location	Date	POF 0	POF 1	POF 2	Total	Spawning Fraction ( $S$ )
1	Scotts Cove	04/02/2011		7	17	90	0.089
2	Scotts Cove	04/02/2011		4	5	86	0.035
3	Scotts Cove	04/02/2011		5	4	89	0.034
4	Scotts Cove	12/02/2011	1	2		89	0.011
5	Scotts Cove	12/02/2011	6	9	4	95	0.067
6	Scotts Cove	12/02/2011	3	5	4	88	0.045
7	St Francis Is.	09/03/2011		1	4	71	0.023
8	Pearson Is.	10/03/2011	4	1	4	96	0.031
9	Pearson Is.	10/03/2011	3		3	91	0.022
10	Pearson Is.	10/03/2011	3		3	92	0.022
11	Flinders Is.	11/03/2011		2	2	32	0.042
12	N. Neptune Is.	12/03/2011	1	7	4	74	0.054
13	N. Neptune Is.	13/03/2011		10	7	90	0.063
14	N. Neptune Is.	13/03/2011	1	8	11	92	0.072
Total			<b>22*</b>	<b>61*</b>	<b>72*</b>	<b>1175*</b>	<b>0.044#</b>

3.5.3 Batch fecundity

Batch fecundity ranged from 6,240 to 29,798 hydrated oocytes for the 19 hydrated female sardines examined in 2011. Based on the relationship (Batch Fecundity = 493.5 x Gonad Free Female Weight – 6274.7,  $R^2 = 0.713$ , Fig. 9) and the mean gonad free female weight (45.2 g) for all samples collected in 2011, mean batch fecundity was 16,049 hydrated oocytes per batch (95% CI = 13,650 – 18,806, Table 5).

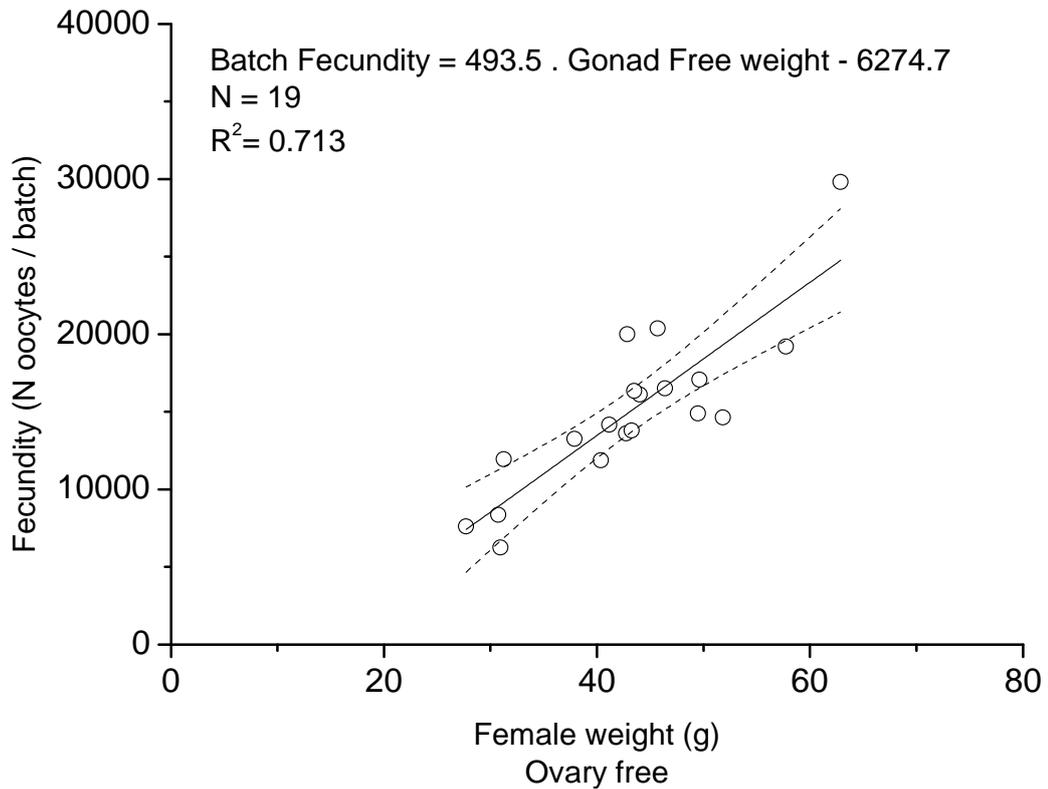


Figure 9. Relationship between gonad-free weight and batch fecundity in 2011 (dotted line = 95% CI).

### 3.5.4 Spawning fraction

Of the 1,175 ovaries examined, 22 had hydrated oocytes and/or day-0 POFs, 61 had day-1 POFs and 72 day-2 POFs (Table 4). The spawning fraction of females in samples ranged from 0.011 to 0.089. The weighted mean spawning fraction for all 2011 data was 0.044 (95% CI = 0.032 – 0.058) whereas the mean value for the period 1998 – 2011 was 0.126 and ranged between 0.044 and 0.179 (Table 5).

**Table 5.** Parameters used in the calculations of spawning biomass, Values for 2011 and the mean, minimum and maximum for the period 1998 to 2011 are presented.

Parameter	2011 (95% CI)	Mean 1998-2011 (min-max)
Egg Production ( $P_o$ , eggs.day <sup>-1</sup> .m <sup>-2</sup> )	44.0 (27.8 – 71.1)	74.0 (38.1 – 120.9)
Sex Ratio (R)	0.63 (0.55 – 0.73)	0.52 (0.36 – 0.63)
Fecundity (F, eggs.female <sup>-1</sup> )	16,049 (13,650 – 18,806)	17,292 (10,904 – 24,790)
Spawning Fraction (S)	0.044 (0.032 – 0.058)	0.126 (0.044 – 0.179)
Female Weight (W, g)	46.4 (42.3 – 51.2)	58.9 (45.2 – 78.7)
Spawning Area (A, km <sup>2</sup> )	42,075	38,499 (14,876 – 53,553)

### 3.6 Re-sampling: Bootstrapping Procedures

The distributions for each variable calculated using 'bootstrap replacement' procedures and the percentile method are shown in Table 5.

### 3.7 Spawning Biomass

The estimate of spawning biomass, calculated using all data from 2011 was 193,201 t (95% CI = 104,151 – 381,961, Table 1, Fig. 10). If the mean sex ratio recorded between 1998-2011 (0.52 (95% CI = 38.0 – 65.9)) was used, spawning biomass increased to 236,209 t (95% CI = 135,924 – 439,280). If the spawning fraction was increased by 50% to 0.066 the spawning biomass decreases to 128,808 t (95% CI = 59,425 – 163,342).

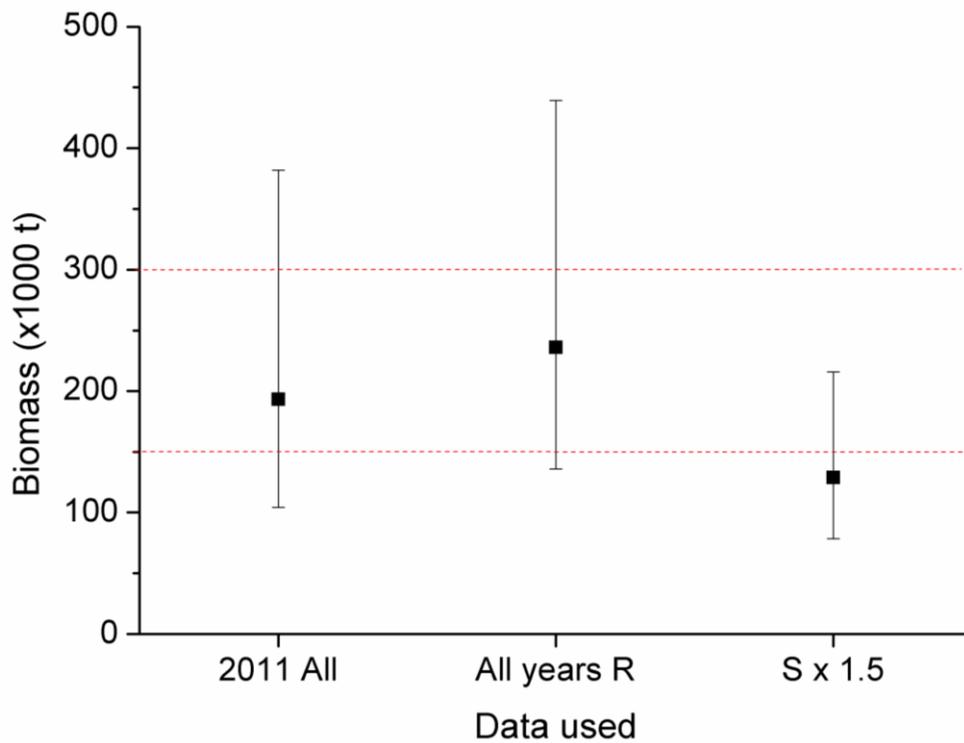


Figure 10. Estimates of spawning biomass obtained using estimate of egg production obtained with the log-linear model and 1), all adult parameters estimated from 2011 samples; 2), sex ratio estimated from 1998 to 2011 samples and 3), spawning fraction at 1.5 times the estimate for 2011. Error bars are 95% confidence intervals, red dotted line is the 150,000 and 300,000 t trigger points for the TACC.

## 4. DISCUSSION

### 4.1 Biophysical variables and egg and larval distribution patterns

The lowest SSTs (<18.0°C) recorded in 2011 were off southern and western Eyre Peninsula indicating the presence of upwelled water along the coast. There was little evidence (low SST) of upwelling around western Kangaroo Island and southern Spencer Gulf. Chlorophyll-a concentrations were low compared to previous years, with no readings higher than 1 µg.L<sup>-1</sup> (e.g. Ward *et al.* 2009c). Most readings above 0.2 µg.L<sup>-1</sup> were recorded near areas of cool water on the western side of Eyre Peninsula. High chlorophyll-a levels were also observed south-east of Kangaroo Island and mid-shelf near the Head of the Bight. Plankton density was generally low compared to other years with the exception of a region of high salp density downstream of the upwelled water off Point Sir Isaac.

The egg samples collected in 2011 (total of 2,796 eggs) were, as is the case in most years, strongly over-dispersed with a few samples containing very high numbers of eggs and many samples without any eggs. Overall, egg densities were generally lower than in recent years (e.g. Ward *et al.* 2009c). Although the number of positive stations was relatively low, stations with eggs were widespread and present in inshore areas between Cape Carnot and Streaky Bay where eggs are often absent in strong upwelling years. This pattern is similar to other years of weak upwelling, such as 2005 (Ward *et al.* 2005). The broad distribution of eggs could indicate wide dispersal of adults in the absence of environmental conditions (e.g. high plankton densities) which are associated with larger more distinct aggregations. The abundance of sardine eggs and larvae in Spencer Gulf, especially north of Wedge Island and in Investigator Straight was low during both February and March in 2011.

From August 2010 onwards, Australia's weather was influenced by the onset of moderate to strong La Niña conditions in the Pacific Ocean after several seasons of El Niño dominance (Bureau of Meteorology 2011). This resulted in a relatively cool wet summer in southern Australia. El Niño events have been shown to be correlated with increased upwelling intensity in the South Australian upwelling regions (Middleton *et al.* 2007). Although SST data collected during the 2011 surveys shows some evidence of usual summertime upwelling, the low chlorophyll-a concentrations and plankton densities indicate the overall level of upwelling may have been low.

### 4.2 Spawning area

The estimate of spawning area for 2011 (42,075 km<sup>2</sup>, Table 1; 5) is within the range recorded over previous years, but is the lowest since 2005 (40,816 km<sup>2</sup>). The distribution of the spawning in 2005 was mainly scattered throughout the western portion of the sampling area, with apparently low levels of spawning activity in southern Spencer Gulf.

### 4.3 Egg production

The estimate of egg production ( $P_0$ ) obtained using the linear version of the exponential egg mortality model, which is the method recommended for sardine in South Australian waters by Ward et al. (2009b), was 44.0 eggs. day<sup>-1</sup>.m<sup>-2</sup> (95% CI = 27.8 – 71.1). This is the lowest egg production since 1999, after the second mass mortality event.

### 4.4 Adult sampling

During the 2011 survey, 14 samples of adult sardine containing 1,175 females were collected from Scotts Cove, St Francis, Pearson Flinders and North Neptune Island. Few fish (one sample, St Francis Island,  $n = 50$ ) were collected west of Pearson Island. This is due to a paucity of locations (i.e. protected bays on islands) that are suitable for sampling adults using the existing methodology (i.e. gill-net and lights). As this western region represents a significant portion of the spawning area, the development of alternative sampling methods which can be used in open water remains a high priority.

Spawning fraction ( $S$ ) during the 2011 was 0.044 (Table 4) which is the lowest recorded in the last 12 surveys (mean value for 1998-2011 is 0.126) and may explain the low egg densities and low estimate of egg production ( $P_0$ ). Spawning fraction was consistently low across all samples collected in 2011, ranging from 0.011 to 0.089.

Several of the adult samples collected during 2011 included a low proportion of males and many non-spawning females, the opposite of the high proportion of males and actively spawning females observed in some samples in 2009 (Ward et al. 2009c). Small ephemeral spawning schools of sardine are known to form immediately prior to, during and after spawning (Statoudakis 2006). These schools are dominated by males and include a high proportion of spawning females (such as the 2009 samples). Conversely, the remainder of the population in non-spawning schools includes a low proportion of males and a few spawning females (such as 2011). Why each type of school was seemingly over represented across multiple samples in 2009 and 2011 is unknown. Although this spawning behaviour can lead to estimates of  $R$  that are biologically unlikely, the effect on estimates of  $S$  is reduced by the method used to estimate  $S$  (Ward et al. 2011; i.e. inclusion all POF stages ( $d 0, 1, 2$ ) in calculation).

Climatic and oceanographic conditions were atypical in the months preceding the 2011 surveys, it is likely that these factors contributed to the low spawning intensity observed. Lower food availability, as indicated by the low plankton abundance and low primary production (Chlorophyll-*a* concentration) can reduce spawning frequency and, in some cases, cause females to forego spawning for an entire season (Rideout et al. 2005). Temperature has also been shown to

influence spawning intensity (Takasuka 2005). Spawning intensity of individual batch spawning fish increases up to the peak of the spawning season and then tapers off (Yamada et al. 1998). It is possible that the 2011 survey missed the peak of the spawning season. However, the simultaneous collection of adult and egg data in the DEPM is designed to ensure that timing issues do not impact on estimates of spawning biomass.

#### **4.5 Spawning biomass estimates**

The estimate of spawning biomass calculated using all data from 2011 was 193,201 t (95% CI = 104,151 – 381,961). As mentioned previously, estimates of two parameters ( $P_o$  and  $S$ ) for 2011 were low compared to recent years (Table 5). This suggests that spawning intensity and, as a result, egg abundance were low during the survey period. Low values for these parameters do not imply a low spawning biomass, just that spawning rates are low. However, estimates of spawning biomass obtained using the DEPM are more precise when spawning rates are high.

#### **4.6 Future research directions**

The best option for improving the precision of estimates of spawning biomass obtained using the DEPM may be to develop a numerical model to integrate data collected by a CUFES and CalVET nets to estimate mean daily egg production. The other opportunity to improve estimates of spawning biomass is to develop alternative adult sampling methods. This would ideally be done in collaboration with members of the SASF and involve investigations to compare estimates of adult parameters obtained by gill-netting, trawling and purse-seining.

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